Docket No.: 1339752-1061352-0052 **PATENT**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	:	Customer Number: 20277
Tetsuo YUKIMASA, et al.	:	Confirmation Number: 7282
Application No.: 10/699,848	:	Group Art Unit: 1637
Filed: November 04, 2003	:	Examiner: PANDE, SUCHIRA

For: METHOD OF DETECTING INORGANIC PHOSPHORIC ACID, PYROPHOSPHATE AND NUCLEIC ACID, AND METHOD OF TYPING SNP SEQUENCE OF DNA

Declaration Under 37 C.F.R. § 1.132

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Hidenobu Yaku, declare as follows:

1. I am a graduate of Nagoya University Graduate School of Bioagricultural Sciences.

2. I am employed as a Senior Researcher by Matsushita Electric Industrial Co., LTD.

3. I am a coinventor of U.S. Patent Application Serial No. 10/699,848, METHOD OF DETECTING INORGANIC PHOSPHORIC ACID, PYROPHOSPHATE AND NUCLEIC ACID, AND METHOD OF TYPING SNP SEQUENCE OF DNA, filed November 4, 2003 (the present invention).

4. I have read and am familiar with the disclosure of the above-captioned patent application.

5. At my direction and under my supervision Sample 4 according to Table 4 was prepared.

Table 4

	Sample 4 Mediator Not Present and Diaphorase Present	
Reagents	Volume	Final Conc.
1 M Tricine(pH 8.8)	9 μl	45 mM
100 mM NAD^+Aqueous Solution	2 μl	1 mM
100 mM Potassium Ferricyanide Aqueous Solution	0 μl	--
0.1 mM Phosphoric Acid Aqueous Solution	160 μl	0.08 mM
30 mM GAP Aqueous Solution	7 μl	1 mM
Distilled Water	12 μl	--
1000 U/ml Diaphorase	2 μl	10 U/ml
Total	192 μl	

6. 96 μl of Sample 4 was introduced into a cuvette and the cuvette and Sample were stored for 5 minutes at 30 °C.

7. 4 μl of 800 U/ml GAPDH was added to Sample 4 to provide a total sample volume of 100 μl with a GAPDH concentration of 32 U/ml.

8. The change in ultraviolet/visible light absorbance of Sample 4 was measured at a wavelength of 340 nm.

9. The phosphoric acid reaction change rate was calculated by dividing the absorbance change rate by the molar absorbance coefficient. The phosphoric acid reaction change rate, absorbance change rate, and the molar absorbance coefficient are shown in Table 5.

Table 5

	Sample 4 Mediator Not Present and Diaphorase Present
Absorbance Change Rate	0.0120 A/min
Measured Wavelength	340 nm
Measured Substance	NADH
Molar Absorption Coefficient	6,220
Phosphoric Acid Reaction Change Rate	1.93 μM/min

10. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further, that these statements are made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this application or any patent issuing therefrom.

January 30, 2007
Date

Hidenobu Yaku
Hidenobu Yaku

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Declaration Under 37 C.F.R. § 1.132

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Tetsuo Yukimasa, declare as follows:

1. I am a graduate of Osaka University, Department of Biophysical Engineering, School of Engineering Science.

2. I am employed as an Engineer by Matsushita Electric Industrial Co., LTD.

3. I am a coinventor of U.S. Patent Application Serial No. 10/699,848, METHOD OF DETECTING INORGANIC PHOSPHORIC ACID, PYROPHOSPHATE AND NUCLEIC ACID, AND METHOD OF TYPING SNP SEQUENCE OF DNA, filed November 4, 2003 (the present invention).

4. I have read and am familiar with the disclosure of the above-captioned patent application.

5. At my direction and under my supervision Samples 1, 2, and 3 according to Table 1 were prepared.

Table 1

	Sample 1 Mediator and Diaphorase Not Present		Sample 2 Mediator Present and Diaphorase Not Present		Sample 3 Diaphorase and Mediator Present	
Reagents	Volume	Final Conc.	Volume	Final Conc.	Volume	Final Conc.
1 M Tricine (pH 8.8)	9 μl	45 mM	9 μl	45 mM	9 μl	45 mM
100 mM NAD^+ Aqueous Solution	2 μl	1 mM	2 μl	1 mM	2 μl	1 mM
100 mM Potassium Ferricyanide Aqueous Solution	0 μl	--	2 μl	1 mM	2 μl	1 mM
0.1 mM Phosphoric Acid Aqueous Solution	160 μl	0.08 mM	160 μl	0.08 mM	160 μl	0.08 mM
30 mM GAP Aqueous Solution	7 μl	1 mM	7 μl	1 mM	7 μl	1 mM
Distilled Water	14 μl	--	12 μl	--	10 μl	--
1000 U/ml diaphorase	0 μl	--	0 μl	--	2 μl	10 U/mM
Total	192 μl		192 μl		192 μl	

6. 96 μl of each Sample was introduced into a cuvette and the cuvettes and Samples were stored for 5 minutes at 30 °C.

7. 4 μl of 800 U/ml GAPDH was added to each Sample to provide a total sample volume of 100 μl with a GAPDH concentration of 32 U/ml.

8. The change in ultraviolet/visible light absorbance of the Samples was measured. Sample 1 was measured at a wavelength of 340 nm. Samples 2 and 3 were measured at a wavelength of 420 nm.

9. The phosphoric acid reaction change rates were calculated by dividing the absorbance change rates by the molar absorbance coefficients. The substrate reaction change rates, absorbance change rates, and the molar absorbance coefficients are shown in Table 2.

10. The reaction between NADH and potassium ferricyanide is a two-electron reaction: NADH + 2 potassium ferricyanide → NAD+ + 2 potassium ferrocyanide. Therefore, the calculated phosphoric acid reaction change rate for Sample 3 was divided by 2 because the ratio of the number of electrons involved in the phosphoric acid:NADH:potassium ferricyanide reaction is 1:1:2.

Table 2

	Sample 1 Mediator and Diaphorase Not Present	Sample 2 Diaphorase Not Present and Mediator Present	Sample 3 Diaphorase and Mediator Present
Absorbance Change Rate	0.0117 A/min	-0.0448 A/min	-1.409 A/min
Measured Wavelength	340 nm	420 nm	420 nm
Measured Substance	NADH	Potassium Ferricyanide	Potassium Ferricyanide
Molar Absorption Coefficient	6,220	1,096	1,096
Phosphoric Acid Reaction Change Rate	1.88 μM/min	20.4 μM/min	643 μM/min

11. As shown in Table 3, Sample 3 containing both diaphorase and the mediator has a phosphoric acid reaction change rate 342 times greater than Sample 1. The relative reaction change rates were determined by setting the reaction change rate of Sample 1 to 1.

Table 3

	Sample 1	Sample 2	Sample 3
Relative Phosphoric Acid Reaction Rates	1	11	342

12. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further, that these statements are made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this application or any patent issuing therefrom.

January 31, 2007
Date

Tetsuo Yukimasa
Tetsuo Yukimasa